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Antimicrobial Susceptibility Testing of *Bacillus anthracis*: Comparison of Results Obtained by Using the National Committee for Clinical Laboratory Standards Broth Microdilution Reference and Etest Agar Gradient Diffusion Methods

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We determined the patterns of antimicrobial susceptibility of 65 isolates of *Bacillus anthracis* (50 historical and 15 recent U.S. clinical isolates) to nine antimicrobial agents using the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution reference method. The results for the 50 historical *B. anthracis* isolates obtained by the broth microdilution method were compared to those generated by the Etest agar gradient diffusion method. One isolate of *B. anthracis* was β-lactamase positive and resistant to penicillin (MIC, 128 μg/ml); a second isolate, which was β-lactamase negative, was borderline penicillin resistant, with the penicillin MICs for the isolate varying from 0.12 to 0.25 μg/ml; and the remainder of the isolates were β-lactamase negative and penicillin susceptible (MICs, ≤0.12 μg/ml). Approximately 78% of the isolates showed reduced susceptibility to ceftriaxone (MICs, ≥16 μg/ml). All *B. anthracis* isolates were susceptible to chloramphenicol (MICs, ≤8 μg/ml), ciprofloxacin (MICs, ≤ 1 μg/ml), clindamycin (MICs, ≤0.5 μg/ml), rifampin (MICs, ≤0.5 μg/ml), tetracycline (MICs, ≤0.06 μg/ml), and vancomycin (MICs, ≤2 μg/ml) by use of NCCLS breakpoints for staphylococci. All 15 recent *B. anthracis* isolates from the United States were susceptible to penicillin, doxycycline, and ciprofloxacin. By use of the susceptibility breakpoint for staphylococci of ≤0.5 μg/ml, 97% of the *B. anthracis* isolates tested would have been categorized as intermediate to erythromycin. No statistically significant difference was found between the results of broth microdilution testing and the results of the Etest method for any of the antimicrobial agents tested; however, the results for penicillin obtained by the Etest were 1 to 9 dilutions lower than those obtained by the broth microdilution method. The differences in the penicillin MICs by the Etest method and the difficulties of reading the Etest results through the glass of a biological safety cabinet may limit the utility of this alternate susceptibility testing method for *B. anthracis* isolates.

Anthrax, which is caused by the gram-positive bacterium *Bacillus anthracis*, is an infectious disease of both humans and animals (7, 16, 17). Most humans are infected through contact with infected animals or contaminated animal products (1, 13, 16, 24). The routes of infection for humans include cutaneous exposure, ingestion, and inhalation. Humans with anthrax most commonly present with the cutaneous form of the disease (13, 17). Naturally acquired inhalational anthrax is rare, as is the gastrointestinal form of disease, the latter of which occurs usually following the consumption of contaminated meat (1, 17). Transmission of *B. anthracis* to humans by contact with infected animals and contaminated animal products is controlled by livestock vaccination programs and the slaughter of potentially infected animals (1).

Recently, the first cases of inhalational and cutaneous anthrax resulting from the intentional release of *B. anthracis* were noted in the United States (3, 14). The antimicrobial chemotherapy recommended for the treatment of patients with inhalational anthrax includes ciprofloxacin or doxycycline plus one or two additional agents (3). Therapy may be switched to penicillin after clinical improvement if the organisms are susceptible to penicillin, as determined by in vitro testing (3, 14). Prior to the discovery of the recent cases of anthrax in the United States, chloramphenicol, gentamicin, and streptomycin were also recommended for the treatment of anthrax (7, 9, 10, 13, 16, 17, 24). Concerns about the therapeutic regimens that should be used for children and pregnant and nursing women with anthrax have been raised since fluoroquinolones and doxycycline are not usually prescribed for the treatment of infections in the pediatric population or nursing mothers (13, 17). These concerns have been addressed in a more recent publication (4).

While vaccines offer some degree of protection against infection with *B. anthracis* (1, 2), in studies with rhesus monkeys, vaccine administration without concomitant administration of an antimicrobial agent did not protect the animals from infection after exposure to a lethal dose of *B. anthracis*, while penicillin, doxycycline, and ciprofloxacin alone were protective for most animals (9). Since widespread vaccination of populations is not feasible at present (1, 7), antimicrobial agents remain the primary therapeutic approach for the treatment of anthrax. However, while some studies have explored the antimicrobial susceptibility patterns of *B. anthracis* strains, stan-

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dardized methods of testing and guidelines for interpretation of results have not been published. The National Committee for Clinical Laboratory Standards (NCCLS) has yet to address the issue of interpretive criteria for *Bacillus* species, including *B. anthracis* (20, 21).

Several groups of investigators have published the results of studies in which they tested the antimicrobial susceptibilities of *B. anthracis* isolates, including Lightfoot et al. (18), who tested the susceptibilities of 70 isolates to nine antimicrobial agents by the agar dilution method. They reported that two isolates were penicillin resistant when a resistance breakpoint of >0.25 μg/ml was used. That breakpoint is 1 doubling dilution above that suggested by both the NCCLS for staphylococci (20) and the British Society for Antimicrobial Chemotherapy for staphylococci and streptococci (28). Both isolates, which apparently originated from the same patient, were β-lactamase positive. However, inconsistencies were noted between the results of β-lactamase testing and penicillin MICs for several other isolates. One isolate was reported to be β-lactamase positive, although the penicillin MIC for the isolate was only 0.03 μg/ml (18). After induction and by use of a 10-fold higher inoculum (1.3×10^7 organisms/ml), the penicillin MIC rose to 64 μg/ml. However, even after induction, the penicillin MICs for some β-lactamase-positive strains remained low. In the same study, by using an apparent resistance breakpoint of ≥8 μg/ml, 69 isolates were reported to be resistant to cefuroxime (18). Doganay and Aydin (8) also tested *B. anthracis* isolates by the agar dilution and disk diffusion methods and, like Lightfoot et al. (18), reported their results as the percentage of isolates that were susceptible, intermediate, or resistant to various antimicrobial agents. However, they did not provide the criteria that they used to define susceptibility and resistance. Another report documenting the appearance of a β-lactamase-positive, penicillin-resistant strain of *B. anthracis* from a cow in France (23) also suggests that β-lactamase production among *B. anthracis* strains is unpredictable since none of the other isolates from the outbreak were β-lactamase positive.

To better understand the antimicrobial susceptibility profiles of *B. anthracis*, we tested 65 *B. anthracis* isolates, including 15 recent isolates from the United States and 50 historical isolates, by the NCCLS broth microdilution reference method and compared the results obtained for the historical isolates of *B. anthracis* by the broth microdilution method to those generated by the Etest agar gradient diffusion method. The Etest method, which is frequently used for susceptibility testing of fastidious organisms, combines the simplicity of disk diffusion testing with the ability to generate a quantitative MIC result (12, 22). The goals of this study were (i) to determine for *B. anthracis* isolates the MICs of commonly used drugs by the broth microdilution reference method and (ii) to evaluate the accuracy of the Etest as an alternate method for the susceptibility testing of *B. anthracis.*

MATERIALS AND METHODS

Bacterial isolates. A total of 65 isolates of *B. anthracis* were included in the study. Fifty *B. anthracis* isolates (30 isolates from humans and 20 isolates from animals) collected between 1937 and 1997 were selected from the strain collection of the Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention (CDC), as representatives of temporally and geographically diverse *B. anthracis* strains. The remaining 15 *B. anthracis* isolates were clinical isolates (defined as an organism isolated from a clinical specimen that had been on an agar plate or slant for less than 7 days and never frozen) collected from the patients recently infected in the United States (3). All isolates were identified by standard microbiological procedures (19), including direct fluorescent-antibody staining and gamma bacteriophage susceptibility testing for the recent isolates. Three quality control strains, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922 (20, 21), were tested daily. The results of the broth microdilution method and the Etest (AB Biodisk, Piscataway, N.J.) for these quality control isolates were within the expected ranges. Prior to testing, all nonclinical isolates were stored at −70°C.

Broth microdilution reference method. Prior to testing, each isolate was subcultured twice on Trypticase soy agar (TSA) plates containing 5% sheep blood (BD BioSciences, Sparks, Md.) that were incubated at 35°C overnight. The susceptibility of each bacterial isolate to nine antimicrobial agents was tested by the NCCLS broth microdilution reference method (20) with 96-well microtiter panels prepared in-house at CDC. The panels were stored at −70°C and thawed at room temperature before use. Growth from a TSA blood agar plate incubated at 35°C for 16 to 20 h was suspended in 5 ml of Mueller-Hinton broth (Remel, Lenexa, Kans.) to the turbidity of a 0.5 McFarland standard. Two milliliters of this suspension was transferred to 38 ml of sterile water. The suspension was inverted 8 to 10 times and then poured into a disposable inoculum tray (Dynex Technologies, Chantilly, Va.). The disposable inoculator delivers approximately 10 μl into a final volume of 100 μl/well. The broth microdilution panels were stacked no more than three high and placed into a self-sealing plastic bag in ambient air at 35°C for 16 to 24 h. The final inoculum was approximately 3×10^4 CFU/ml, as determined from the colony counts for the growth control well.

Etest method. The Etest method was performed as described by the manufacturer. The suspension with a turbidity of a 0.5 McFarland standard prepared for each isolate was also used to inoculate two Mueller-Hinton II 150-mm agar plates (BD BioSciences) with a sterile swab. After 10 min, four Etest strips were placed on one Mueller-Hinton II agar plate and five Etest strips were placed on the other. The plates were inverted, stacked no more than two high, and incubated in ambient air at 35°C for 16 to 24 h.

β-Lactamase testing. β-Lactamase testing was performed by using a commercial nitrocefin product (Dryslide Nitrocefin; BD BioSciences) as described by the manufacturer. The inoculum was taken from a 16- to 20-h-old TSA plate. The reaction mixtures were held for 1 h before being interpreted as negative.

Interpretation of results. The breakpoints used to determine susceptible, intermediate, and resistant are given in Table 1. The results of the broth microdilution reference test and the Etest were read at 16 and 24 h. Adequate growth was observed at 16 h for all organisms, and thus, the results obtained at 24 h were disregarded. The result for any isolate for which the result of the Etest was recorded as 2 dilutions higher or lower than the result of the broth microdilution reference method was reexamined to control for reading and clerical errors. Isolates for which the MIC results differed by more than 1 doubling dilution between the Etest and the broth microdilution reference method were retested in duplicate. The results were recorded and the data were entered into an Epi Info database. The data set was later converted to an SAS (version 6.12; SAS Institute, Inc., Cary, N.C.) data set for analysis. The Wilcoxon signed-rank test was performed to determine trends in discrepancies in MICs obtained by the broth microdilution reference method and those obtained by the Etest method. A P value of ≤0.05 defined a statistically significant association.

Safety. Due to the potential aerosol hazard, all antimicrobial susceptibility tests were performed in a biosafety level 3 (BSL 3) laboratory within a class II biological safety cabinet and in full compliance with all safety procedures recommended for work in a BSL 3 laboratory (26). Specifically, laboratory personnel received the anthrax vaccine adsorbed series prior to initiation of the study. In addition, personnel used protective clothing including surgical scrub suits worn over their own clothing, disposable shoe covers, disposable laboratory coats, and two pairs of gloves as well as a powered air-purifying respirator. Care was taken to avoid disruption of the inward directional airflow into the safety cabinet by moving slowly within the BSL 3 laboratory and by restricting movements inside the biological safety cabinet. Only six samples of *B. anthracis* were tested daily. Etest and broth microdilution reference test results were viewed through the glass of the biological safety cabinet. Copies of the work cards containing the results were sent by fax from the BSL 3 laboratory to an office outside of the BSL 3 area. All waste materials, including paper, were autoclaved before removal from the laboratory.

RESULTS

The concentration that inhibited the growth of 50% of the isolates (MIC_{50}), the MIC_{90}, and the MIC range of each an-

TABLE 1. MIC_{50}s and MIC_{90}s for 65 *B. anthracis* isolates tested by reference broth microdilution method

Antimicrobial agent	MIC (μg/ml)			% of isolates with the following categorical interpretation[a]			Staphylococcal breakpoints (μg/ml)[b]		
	50%	90%	Range	S	I	R	S	I	R
Ceftriaxone	16	32	4–32	22	78		≤8	16–32	≥64
Chloramphenicol	4	4	2–8	100			≤8	16	≥32
Ciprofloxacin	0.06	0.06	0.03–0.12	100			≤1	2	≥4
Clindamycin	≤0.5	1	≤0.5–1	94	6		≤0.5	1	≥2
Erythromycin	1	1	0.5–1	3	97		≤0.5	1–4	≥8
Penicillin	≤0.06	≤0.06	≤0.06–128	97		3	≤0.12		≥0.25
Rifampin	≤0.25	0.5	≤0.25–0.5	100			≤1	2	≥4
Tetracycline	0.03	0.06	0.03–0.06	100			≤4	8	≥16
Vancomycin	2	2	0.5–2	100			≤4	8–16	≥32

[a] S, susceptible; I, intermediate; R, resistant.
[b] Data are from reference 21.

timicrobial agent for the 65 *B. anthracis* isolates tested are shown in Table 1. Categorical interpretations (susceptible, intermediate, and resistant) for *B. anthracis* have not been established by NCCLS; therefore, based on the types of infections caused by *B. anthracis* and the distributions of MIC results observed for the various antimicrobial agents tested, we used the breakpoints for staphylococci for chloramphenicol, ciprofloxacin, erythromycin, penicillin, rifampin, tetracycline, and vancomycin and the general breakpoints for nonfastidious organisms for ceftriaxone (21). The penicillin breakpoints for staphylococci are within a doubling dilution of those previously used by Lightfoot et al. (18). By using the breakpoints for staphylococci, two *B. anthracis* isolates were initially classified as penicillin resistant (MICs, ≥0.25 μg/ml); however, one isolate with a borderline result (MIC, 0.25 μg/ml) had a result in the susceptible range (MIC, 0.12 μg/ml) when it was retested. The reproducibly resistant strain (MIC, 128 μg/ml) was the only β-lactamase-positive *B. anthracis* isolate noted in the study. This highly penicillin-resistant strain was one of the isolates that Lightfoot and colleagues (18) previously found to be resistant. Seventy-eight percent of the *B. anthracis* isolates that we tested were classified as intermediate to ceftriaxone (MICs, 16 μg/ml). All *B. anthracis* isolates were susceptible to chloramphenicol, ciprofloxacin, rifampin, tetracycline, and vancomycin (Table 1). By using the staphylococcal susceptibility breakpoint for erythromycin (≤0.5 μg/ml), 97% of the *B. anthracis* isolates were categorized as intermediate. That categorization would have been changed to 100% susceptible if the breakpoint had been moved 1 dilution higher to ≤1 μg/ml. No data are available from studies with clinical or animal models to indicate which interpretation is correct.

The 50 historical *B. anthracis* isolates were also tested for antimicrobial resistance in parallel by the Etest method (Table 2). For 45 of the isolates, the penicillin MICs obtained by one or both methods were less than or equal to the lowest dilution tested, limiting the number of on-scale comparisons that could be made. Off-scale values were less of a problem with the other antimicrobial agents tested. Tests were repeated for five isolates for which the MICs obtained by the broth microdilution reference method and the Etest method differed by >1 doubling dilution. These included the result for ceftriaxone for one isolate and the results for penicillin for four isolates. In general, the penicillin MICs obtained by the Etest method were lower than those obtained by the broth microdilution reference method. On initial testing, the MIC for the penicillin-resistant strain obtained by the Etest was 9 $\log_2$ dilutions lower than that

TABLE 2. Comparison of Etest MIC results to broth microdilution MIC results for antimicrobial agents tested against 50 *B. anthracis* isolates

Antimicrobial agent	No. of isolates with the following dilution difference in Etest MICs compared to MICs obtained by the broth microdilution reference method:							% Agreement[a]	*P* value[b]	% Agreement after retesting[a]
	≤−3	−2	−1	0	+1	+2	≥+3			
Ceftriaxone	0	1	1	23	25	0	0	98	0.159	100
Chloramphenicol	0	0	21	28	1	0	0	100		100
Ciprofloxacin	0	0	0	42	8	0	0	100		100
Clindamycin	0	0	4	46	0	0	0	100		100
Erythromycin	0	0	26	24	0	0	0	100		100
Penicillin	3[c]	1	1	45	0	0	0	92 (20)[d]	0.047	96 (33)
Rifampin	0	0	5	45	0	0	0	100		100
Tetracycline	0	0	5	44	1	0	0	100		100
Vancomycin	0	0	1	32	17	0	0	100		100

[a] Excluding off-scale values.
[b] By the Wilcoxon signed-rank test.
[c] Etest results were 1 to 9 doubling dilutions lower.
[d] Only five pairs of MIC results were on scale and available for comparison; the percent agreement for those five pairs is given in parentheses. The results for two pairs were resolved on retesting. The *P* value (by the Wilcoxon signed-rank test) for the penicillin MIC results after retesting was 0.16 (nonsignificant).

obtained by the broth microdilution method. The penicillin MICs for three other *B. anthracis* strains obtained by the Etest were also 2 to 4 doubling dilutions lower than those obtained by the broth microdilution method, although the MICs were in the susceptible range. Only two of these discrepant results were resolved during retesting.

In addition to ciprofloxacin, we tested the susceptibilities of a subset of 20 *B. anthracis* isolates, including 15 historical isolates and 5 recent clinical isolates, to eight additional fluoroquinolones. All of the isolates were susceptible to clinafloxacin (MICs, ≤0.03 μg/ml), gatifloxacin (MICs, ≤0.12 μg/ml), gemifloxacin (MICs, ≤0.06 μg/ml), levofloxacin (MICs, ≤0.12 μg/ml), moxifloxacin (MICs, ≤0.12 μg/ml), ofloxacin (MICs, ≤0.25 μg/ml), sparfloxacin (MICs, ≤0.25 μg/ml), and trovafloxacin (MICs, ≤0.12 μg/ml) by the broth microdilution reference method. We also tested the susceptibilities of these 20 isolates to clarithromycin, azithromycin, and doxycycline. The MIC ranges were 0.12 to 0.25, 1 to 2, and ≤0.015 μg/ml, respectively.

DISCUSSION

Although previous studies have examined the susceptibilities of *B. anthracis* isolates to various antimicrobial agents (8, 18), no standardized method of testing and no interpretive criteria have been established for these organisms. We found that the use of unsupplemented Mueller-Hinton broth in 96-well plates incubated at 35°C in ambient air for 16 to 20 h produced results that were easy to read, even through the glass of a biological safety cabinet. Because of the possibility that highly virulent, multidrug-resistant organisms may be encountered, we believe that it is important to use BSL 3 facilities to perform susceptibility testing procedures with *B. anthracis*. Overall, with the exception of the penicillin MICs, we found that the antimicrobial susceptibility profiles for the 65 *B. anthracis* strains were consistent. The MICs of penicillin, chloramphenicol, ciprofloxacin, and erythromycin that we obtained by the broth microdilution reference method are consistent with those obtained by the agar dilution method by Lightfoot et al. (18) and Doganay and Aydin (8), who tested 70 and 27 *B. anthracis* isolates, respectively. Lightfoot et al. (18) defined a penicillin MIC of >0.25 μg/ml (equivalent to ≥0.5 μg/ml) as the breakpoint for resistance. This breakpoint is 1 doubling dilution higher than the breakpoint for penicillin resistance for staphylococci published by NCCLS (21) and the breakpoint published by the British Society for Antimicrobial Chemotherapy (28) in 1988 for staphylococci and streptococci. Lightfoot et al. (18) reported that two isolates were penicillin resistant, although both isolates were from the same patient. The penicillin MIC reported was 64 μg/ml, and the strain was a constitutive β-lactamase producer. The same strain was included in our study and was also penicillin resistant (MIC, 128 μg/ml) and produced β-lactamase. However, retesting of this isolate five times over a period of 2 weeks yielded penicillin MICs that ranged from a low of 4 μg/ml to 128 μg/ml. This range may be due to the effect of slightly different inoculum sizes, the age of the subculture used to prepare the inoculum, the incomplete induction of β-lactamase, or other less well defined factors, as noted in the study of Lightfoot and colleagues (18). The strain remained β-lactamase positive regardless of the MIC result. Although both Lightfoot et al. (18) and Doganay and Aydin (8) reported that their isolates were resistant to expanded- and broad-spectrum cephalosporins, they did not provide specific interpretive criteria. Since Lightfoot et al. (18) reported that the range of cefuroxime MICs was 4 to 32 μg/ml and that only a single isolate was susceptible to cefuroxime, we can assume that their criteria for resistance was a cefuroxime MIC ≥8 μg/ml. If we extrapolate those criteria to ceftriaxone, 98% of our isolates would also be considered resistant rather than intermediate to ceftriaxone. Categorization of these isolates as "nonsusceptible" is key, as the MICs indicate that extended-spectrum cephalosporins would be poor choices for the treatment of inhalational anthrax.

The ciprofloxacin MICs were 0.03 to 0.12 μg/ml for all 65 *B. anthracis* isolates tested, which would be interpreted as susceptible by use of the NCCLS breakpoints for staphylococci and other nonfastidious organisms (21). Ciprofloxacin was selected by the Working Group on Civilian Biodefense as one of the primary agents for postexposure prophylaxis of adults, including pregnant women, and children (13), a position that was reiterated in recent CDC recommendations (3). Our in vitro MIC results support the effectiveness of this antimicrobial agent. Although there are normally contraindications to the use of fluoroquinolones by children and pregnant women (7, 13, 17), the risks posed by an intentional release of *B. anthracis* spores outweigh the other risks (4), particularly given the data from studies with animals showing the effectiveness of ciprofloxacin as postexposure prophylaxis (9). We tested the activities of several other fluoroquinolones against a subset of historical and recent clinical strains and found that they were all highly active. On the basis of a presumed mechanism of resistance involving alterations in DNA gyrase or topoisomerase IV, we assume that low ciprofloxacin MICs could be used to predict susceptibility to the other fluoroquinolones. Although Choe et al. (5) have demonstrated that the ofloxacin MICs for the *B. anthracis* Sterne strain can be increased from 0.2 to 0.8 μg/ml by continuous passage in vitro, this MIC still remains within the susceptible range defined for nonfastidious organisms. Doxycycline, which was listed in the CDC recommendations as the other primary therapeutic agent for the treatment and prophylaxis of inhalational anthrax (3), was also highly active against the *B. anthracis* strains in our study (MICs, ≤0.015 μg/ml). The attempts of Choe et al. (5) to increase the doxycycline MIC were unsuccessful.

In older literature, erythromycin was suggested as an alternative treatment for children and pregnant women (7), but 97% of our isolates showed reduced susceptibility to erythromycin (the results were in the intermediate range) when the breakpoint for staphylococci of ≤0.5 μg/ml was used. If the breakpoint is elevated 1 doubling dilution, all of our isolates would have been considered susceptible. In contrast, all of the results for clarithromycin and azithromycin were in the susceptible range when the staphylococcal breakpoints for these drugs were used (21). Clarithromycin in particular was among the secondary agents suggested for use in combination with either ciprofloxacin or doxycycline (3). Interestingly, Lightfoot et al. (18) reported their isolates for which erythromycin MICs were 1 μg/ml as susceptible. We are not aware of any data from studies with clinical or animal models that indicate which erythromycin MIC interpretation would best correlate with

clinical outcome. Our in vitro results also suggest that chloramphenicol and vancomycin in combination with ciprofloxacin or doxycycline may be alternative choices for the treatment and prophylaxis of *B. anthracis* infections; however, no clinical data that support their effectiveness are available at this time.

Other *Bacillus* species, such as *B. cereus*, *B. subtilis*, and *B. thuringiensis*, tend to be more resistant to antimicrobial agents than *B. anthracis* strains (6, 25, 27). Thus, it is possible that resistance may become a more prominent feature of *B. anthracis* strains in the future. Thus, susceptibility testing will continue to play a key role in the management of anthrax infections (13, 15).

While the results of the Etest proved to be comparable statistically to those of the broth microdilution reference method for the testing of the MICs for *B. anthracis*, caution should be exercised when this method is used, particularly for the determination of penicillin resistance. The penicillin-resistant *B. anthracis* isolate was reproducibly categorized as borderline penicillin susceptible (MICs, 0.19 to 0.25 μg/ml) by the Etest method. Although the penicillin MIC result of 0.19 μg/ml would be considered to indicate resistance if it was rounded up to the next doubling dilution (i.e., 0.25 μg/ml), the differences in the MICs (4 to 9 doubling dilutions in multiple tests) are a cause for concern. Most *B. anthracis* isolates produced a well-defined ellipse of inhibition around the Etest strip; however, reading of the MIC through the window of the biological safety cabinet was very difficult. Single colonies or a haze within the ellipse, which might indicate emerging resistance, would be difficult to ascertain through that window.

In those instances in which NCCLS has yet to define criteria for interpretation of MIC results for a bacterial species, it is usually recommended that the MIC results be reported without interpretation, particularly for fastidious organisms (11). However, given that (i) *B. anthracis* is not a fastidious organism, (ii) there is a significant amount of literature on the pharmacokinetics and pharmacodynamics of the key antimicrobial agents that would be used for the treatment of anthrax, (iii) the sites of infection for *B. anthracis* and *S. aureus* are similar, (iv) there is a need for public health agencies to provide timely guidance to physicians caring for patients with *B. anthracis* infections, and (v) the resistance breakpoints for the antimicrobial agents tested are the same for *S. aureus* and for most nonfastidious organisms (21), we selected the interpretive breakpoint criteria for staphylococci to provide preliminary guidance to physicians regarding the interpretation of the MIC results for the *B. anthracis* strains from the recent anthrax cases in the United States (3). The breakpoints for staphylococci were also similar to those previously chosen by Lightfoot et al. (18). These interpretive criteria have yet to be validated by the NCCLS, but they serve as a starting point for such discussions.

There is much to learn about the mechanisms of antimicrobial resistance in *B. anthracis*, particularly about intrinsic β-lactam resistance. On the basis of data from the study of Lightfoot et al. (18) and our own studies, β-lactamase testing with nitrocefin does not appear to be a suitable surrogate for penicillin MIC testing to predict penicillin resistance. It is hoped that these MIC data can serve as a foundation for additional investigations.

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